THE ORDER OF APPEARANCE OF THE ANTERIOR SOMITES IN THE CHICK.[1]

J. THOS. PATTERSON.

A. Introduction.

The statement that in the chick, somites arise in front of the first somite formed in the series has been widely accepted by embryologists. This view, nevertheless, is not in accord with our knowledge concerning the early development of birds, for it is well known that differentiation usually begins at the anterior end and progresses posteriorly.

Although workers in this field agree that somites arise anterior to the one first formed, yet they differ as to the exact number. Thus Balfour ('85) states that there is one, while His ('68) and von Baer ('28) have estimated it at two. Kupffer and Benecke ('79) would lead one to believe that there were at least three or four. So far as I am aware the latest work done to determine this number is by Miss Platt ('89), who concludes from a study of sections that there are two, or, to be more exact, one and a half.

From the results of certain experiments, made in connection with an experimental study of the early development of the pigeon, the writer was led to believe that *no* somites were formed in front of the first mesodermic cleft, except, of course, the so-called rudimentary or incomplete anterior cephalic somite. At the suggestion of Professor Lillie I have performed a number of experiments to test the validity of this view. These experiments, in connection with others, were conducted on a farm in Ohio, where I had at my disposal the eggs from fifty laying hens. It was possible, therefore, to collect and incubate the eggs hourly.

It gives me pleasure here to express my thanks to Professor Lillie for his kindness in sending all the necessary equipment for this work from these laboratories, and for his valuable criticisms.

[1] Unless otherwise indicated, the word somite will be used throughout this work to mean protovertebra.

B. Methods.

For opening and sealing the egg I have in the main employed the method first used by Miss Peebles ('98). By the aid of a fine file a small window is made in the shell just above the blastoderm. The operation is then performed and the opening closed with a slightly larger piece of shell (with membrane still attached) from the corresponding part of a fresh egg.

Although I have used very fine glass pins in some of the work, yet I have found the electric needle by far the better means for making the injury. These needles (No. 12 sewing needles, ground as fine as possible on a water stone) were connected with two dry battery cells.[1] Then, by the aid of a binocular, using a combination of lenses giving a magnification of 12.6 diameters, one needle is placed at the desired point and the other is touched to the albumen for a second or two. The opening is then closed in the manner stated above. The whole procedure from the opening to the closing of the egg need not take over a minute.

If no further precautions were necessary the experimental work would be a simple process, but the difficulties that attend experimental studies on the bird's egg are many. Perhaps there is none so perplexing as that of preventing infection. Previous workers have realized this fact. Some writers have reported a loss of embryos, due to mould or bacteria, reaching as high as 80 per cent.

The mere heating of the instruments is not sufficient in itself to prevent infection. However, I find that if one uses a .1 per cent. solution of bichloride of mercury previous to heating, this difficulty is practically overcome. The table upon which the operation is performed, the hands, the instruments, in fact, every thing connected with the operation must be thoroughly washed in this solution. With a cloth moistened in the sublimate I also wipe off the shell where the window is to be made, otherwise small fragments of the shell falling upon the albumen will be a frequent source of infection.

[1] The Cleveland Dry Battery Cells were used. Each cell has a pressure of about 1.3 volts.

[2] All the drawings with which this paper is illustrated were made by the aid of the Abbe camera.

After closing the opening I place over it a piece of sterilized cotton about 5 cm. square and holding down the edges of the cotton on the sides of the shell, slowly revolve the egg until the closed window is on the lower side. It is then placed in a watch-glass and incubated the desired period of time. In addition to holding the piece of shell in place, the cotton prevents the egg from rolling and facilitates handling.

Inverting the egg serves a double purpose. In the first place, no matter how careful one may be a certain number of eggs are sure to become infected by germs falling on the albumen from the air. Now, when the egg is revolved, the yolk turns until the blastoderm is uppermost and hence removed as far as possible from the region of possible infection, which spreads too slowly to reach the blastoderm and interfere with the development of the embryo, especially if the egg is incubated but a few hours. In the second place, by revolving the egg the blastoderm is brought into an environment almost, if not entirely, normal. Mitrophanow ('97) has shown that varnishing the shell above the blastoderm retards development by limiting the supply of oxygen, and produces abnormalities. A similar effect is undoubtedly produced by placing an extra piece of shell above the embryo and sealing down its edges with strips of membrane — a method used by some workers. Any abnormalities thus produced are to be avoided, because they complicate the correct interpretation of one's experimenal results.

In order to test whether inverting the egg brings the blastoderm into a normal environment, some eggs were thus turned, while others were allowed to remain with the covered window uppermost, or turned but slightly to one side. In general, the latter were delayed from two to four hours, while the former developed equally with the controls.

It may seem that the above precautions are a bit tedious and unnecessary, but one is fully repaid for the trouble thus taken, as shown by the following statistics. During the period in which this series of experiments was carried on over 400 operations were made and but five eggs were infected, or less than 2 per cent., and during the present year about 100 operations have been performed with not a single case of infection noted. Of

this entire number between 80 and 90 per cent. of the eggs have given definite results.

C. Experiments.

If only the rudimentary somite arises in front of the first mesodermic cleft, an injury made just anterior to this cleft ought to destroy, at least partially, this incomplete somite; but if, in addition, complete somites are formed anterior to this cleft, the injury ought to appear later in that one of the complete somites lying just in front of the cleft.

In performing such an operation there are two sources of difficulty. In the first place, owing to the individual variation in the early development of eggs, it is very difficult to hit upon the exact time when but one cleft is present. This difficulty can be met by opening the egg one to two hours before the cleft ordinarily appears and temporarily sealing the opening, so that it may be reopened and examined from time to time until the cleft appears. It was found that the average time of appearance of this cleft is between 21 and 22 hours. In the second place, it is almost impossible in a large number of embryos, to see the cleft so that one may be sure of the operation. This is due to the fact that the yolk is often a very pale yellow, and the white embryo cannot be seen. However, in about 20 per cent. of the eggs the yolk is a deep yellow, and against this background the embryo stands out in perfect contrast, and one can be absolutely certain of one's operation. In this work only the latter kind of eggs was used.

Experiment I.

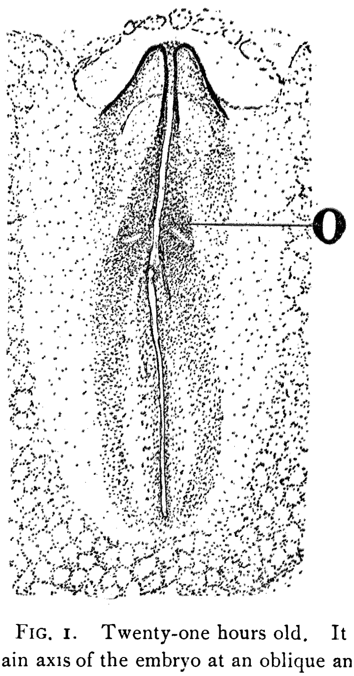
The operation was performed with an electric needle after the egg had been incubated 22 hours at a temperature of 37–39° C. Fig. 1 illustrates the place of injury (Fig. 1, *O*) and the condition of the embryo at the time of the operation. It will be noted that the first cleft lies just anterior to the fore-end of the primitive streak and meets the main axis of the embryo at an oblique angle.

After the operation the egg was incubated ten hours. The embryo was killed in picrosulphuric-acetic acid, stained in Conklin's picro-hæmatoxylin, and mounted in xylol-balsam.

Fig. 2 shows the result of the operation. On the right side the rudimentary somite is greatly disturbed (Fig. 2, *O*) and the

neural tube is slightly injured. Aside from this the embryo is normal in every way. The eighth pair of somites is just being cut off and the heart is forming.

A sagittal section through the somites of this embryo confirms



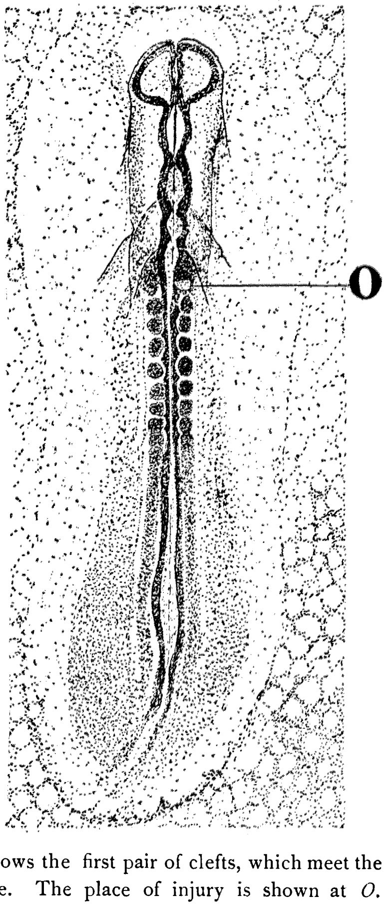


FIG. 1. Twenty-one hours old. It shows the first pair of clefts, which meet the main axis of the embryo at an oblique angle. The place of injury is shown at *O*. × 20.

FIG. 2. Twenty-two hours old when operated on, and then incubated ten hours. It shows eight pairs of somites, and the injury in the right anterior somite at *O*. × 20.

what is seen in surface view. The rudimentary somite is greatly injured (Fig. 3, *x*) and its characteristic enlargement (see Figs. 10–12) is not present.

Experiment II.

The conditions under which the operation was performed and the subsequent handling of the embryo were the same as in the preceding experiment. Instead of using the electric needle, a very fine glass "pin" was substituted. This pin was placed just in front of the first cleft on the left side at a stage corresponding to that of Fig. 1. After the operation the egg was incubated twenty and one half hours longer.

The result of the operation is shown in Fig. 4. The needle is found in the incomplete somite on the left side (Fig. 4, *O*). There are 16 pairs of somites and the heart is well formed. It was im-

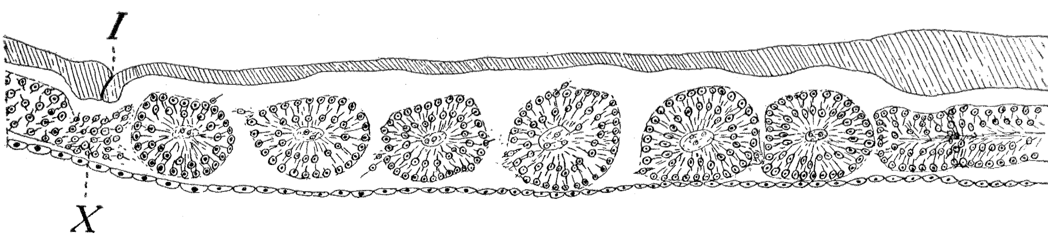


FIG. 3. Sagittal section through the somites on the right side of the embryo represented in Fig. 2. It shows at *X* the destroyed incomplete somite and at *I* the place where the needle has broken through the ectoderm. × 157.

possible to section this embryo on account of the glass pin, but the surface view is so clear that there can be no doubt as to the position of the pin.

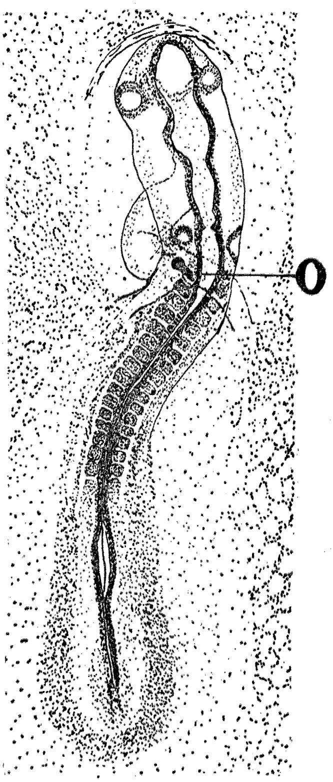
Experiment III.

In order to show that the mesoderm lying between the injury and the cleft (between *r* and *a*, Fig. 6) does not increase subsequent to the operation and antecedent to the examination of the result and give rise to one or more somites, the injury was made so as to destroy the mesoderm spanning the first cleft (Fig. 6, *a*).

The result of such an experiment is well illustrated in Fig. 5. This embryo was treated in exactly the same manner as the one represented in Fig. 2. In addition to destroying the posterior edge of the incomplete somite, the injury extends over a portion of the cleft (Fig. 5, *O*). In this, as in Fig. 2, sections confirm what is seen in surface view.

The above are only a few types out of about seventy-five ex-

periments performed to throw light on the order of development of the somites. In all cases the results support the view that only the incomplete somite arises anterior to the first mesodermic cleft. In the cases cited, the oldest embryo was carried to the sixteen-somite stage, but other embryos were allowed to de-



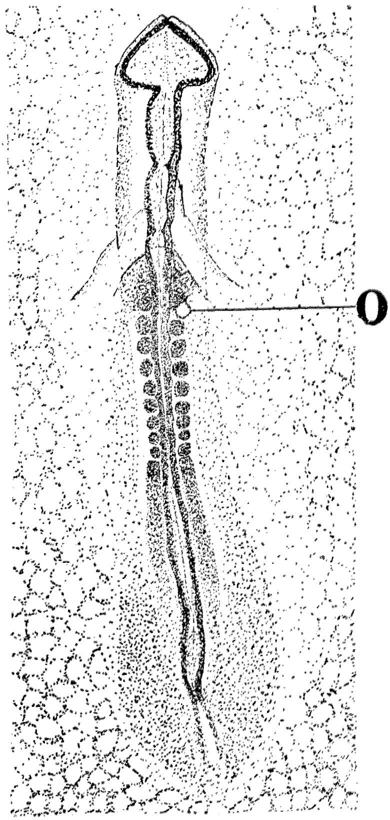


FIG. 4. Twenty-two hours old when operated on, and then incubated twenty and one half hours. There are sixteen pairs of somites. The glass pin is located in the incomplete somite on the left side, at *O*. $\times$ 20.

FIG. 5. Twenty-two hours old when operated on, and then incubated ten hours. The injury is shown at *O*, and ten pairs of somites are present. $\times$ 20.

velop until twenty or twenty-five somites appeared, with the injury still found in the rudimentary somite. In another series the injury was made just posterior to the first cleft. In such cases the first complete somite was either greatly disturbed or entirely destroyed.

D. Study of Sagittal Sections.

The results of the above series of experiments are in themselves sufficient to prove the position taken in this paper. But inasmuch as some writers have drawn their conclusions from a study of sections, it seems advisable to introduce a few figures to show that sections support the above contention. By far the

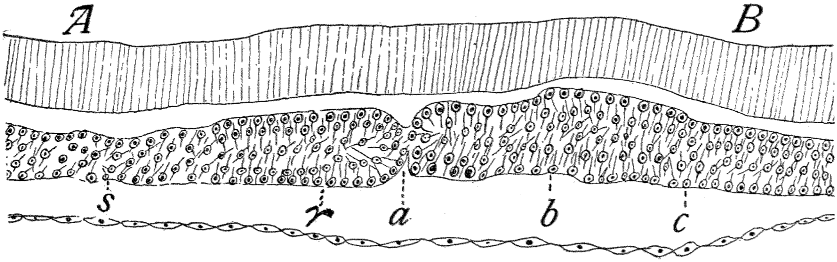


FIG. 6. Shows the first mesodermic cleft, and there are also indications of the second and third clefts. *r*, beginning of the rudimentary somite. × 247.

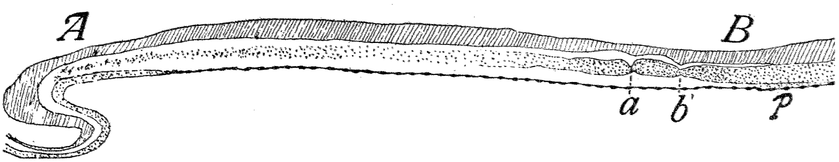


FIG. 7. Introduced to show the relation of the first and second clefts to the rest of the embryo. *p*, anterior end of the primitive streak; *b–p*, region of differentiation of the embryo. × 68.

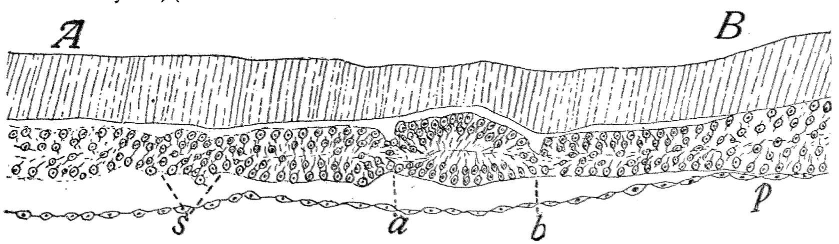


FIG. 8. Enlarged portion of the opposite side of the embryo represented in Fig. 7. *s*, shallow depression. × 247.

FIGS. 6–8. In these, as in the remaining figures of this paper, *A* is anterior and *B* posterior end, and *a*, *b*, *c*, etc., are respectively the first, second, third, etc., clefts.

most critical study made on sections is by Miss Platt, whose view is well summed up in her conclusion, in which she says: "My conclusions are, therefore, that the first break in the mesoderm occurs anterior to the first protovertebra, and that two protovertebræ (or, more correctly, one and a half) are slowly formed

anterior to the first mesodermic cleft, in the time occupied by the formation of six or seven protovertebræ posterior to that cleft."[1]

The first indication of somites is a depression on the dorsal surface of the mesoderm lying at the sides of the notochord, just anterior to the fore-end of the primitive streak. This is soon followed by a corresponding indentation on the under side (Figs. 6 and 7, *a*). Between the cleft and the anterior end of the primitive streak, indications of the two succeeding clefts are already present (Fig. 6, *b* and *c*). In front of the cleft, the strip of mesoderm which extends forward into the head gradually thins out anteriorly. At some points this thinning out has progressed more rapidly than at others, giving rise to shallow, transitory depressions (Figs. 6 and 8, *s*), which have been wrongly interpreted as clefts by Miss Platt.

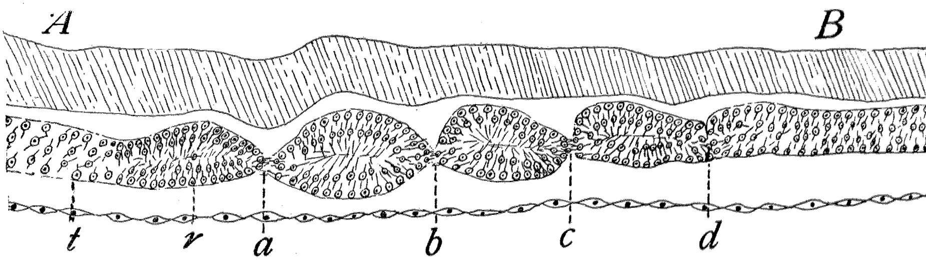
The mesoderm immediately anterior to the first cleft is destined to form the rudimentary somite, and the manner in which this structure arises can be followed with no small degree of certainty. At first the mesoderm is quite uniformly thick (Fig. 9, *t*), but by the time three or four somites are formed its posterior edge has become much enlarged. Apparently this thickening takes place at the expense of the mesoderm just anterior to it (cf. Figs. 9–11, *t*). It should be remembered, however, that this incomplete somite is never so large as the others, really being, as Miss Platt states, only a half-somite. It must be considered as a part of the head mesoderm, from which it never becomes separated (Figs. 9–12, *r*).

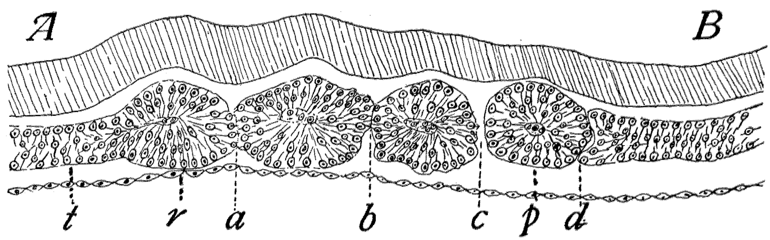
Miss Platt used the relative depths of the clefts as a means for determining the priority of somites. After very justly criticising Kupffer for judging either the fourth or fifth somite to be the oldest on account of its size, she says: "I think enough has been said to show that neither the size of the protovertebræ, their relative distance from the primitive streak, nor yet their obliquity to the main axis, is a sufficient ground to warrant a decisive answer to the question in regard to the order of their development,"[2] and I should add, that neither can the depth of the cleft be taken as a criterion for ascertaining seniority. The error into which one

[1] *Loc cit.*, p. 178.
[2] *Loc. cit.*, p. 174

falls by using such an index, becomes apparent on examination of sections such as are represented in Figs. 9 and 10. In Fig. 9, clefts *a*, *b*, and *c* are so nearly alike that it would be impossible to say which is the oldest, judging from their depths.

In Fig. 10 cleft *b* is slightly deeper than *a*, but *c* is still deeper. In such cases, those who accept the relative depths of the clefts as a criterion for drawing conclusions, would be forced to say that two and one half somites arise anterior to the first formed somite, because, as Miss Platt correctly states, "the first cleft lies





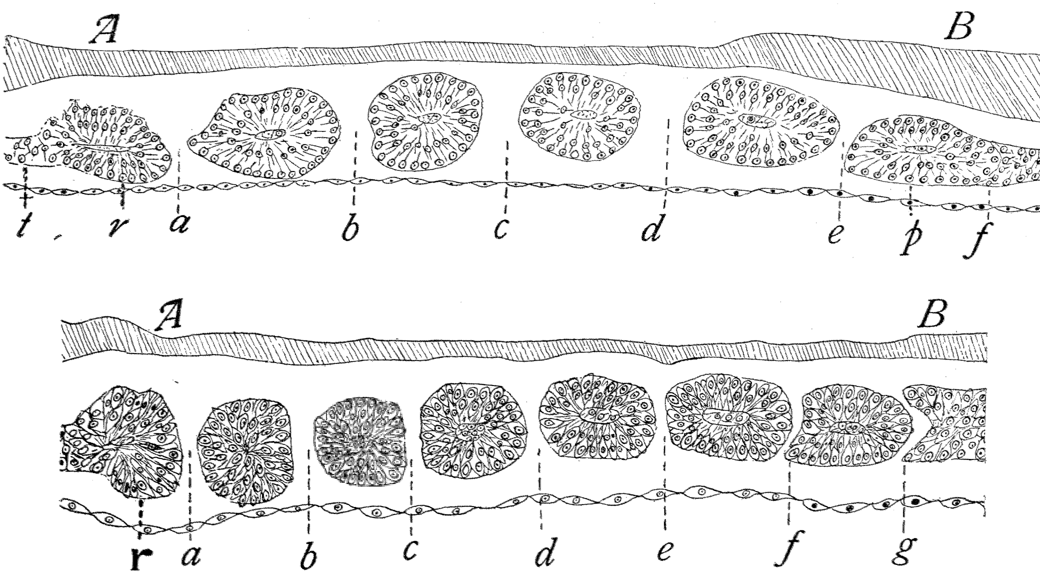
FIGS. 9 and 10. Sections of three and four somites respectively. Both figures show the failure of the first clefts to cut off completely the anterior somites in the beginning. × 157.

anterior to the first protovertebra, not posterior, as Kupffer and Benecke supposed."[1] In other cases I have observed the fourth cleft to be the deepest.

The condition seen in Fig. 10 is a very common one, and is brought about by the manner in which somites posterior to the first two or three are cut off. In the beginning the first clefts never completely separate their bordering somites, so that the anterior somites often remain connected until the sixth or seventh pair is formed, but posterior to these anterior clefts, succeeding somites are delimited, often before there are any indentations on

[1] *Loc. cit.*, p. 176.

the upper or under surface of the mesoderm (Fig. 11, *f*). As seen in section, the forming somite is at first elongated, with its long axis coinciding with that of the embryo (Fig. 11, *p*). However, it soon rounds up (Fig. 10, *p*), and in so doing becomes separated from the posterior mesoderm (Fig. 12, *g*). The cleft thus made is never vertical, but is so formed that the posterior edge of the last formed somite is in the shape of a wedge, which fits into a corresponding concavity on the anterior edge of the unsegmented mesoderm (Figs. 10–12). That the above process



FIGS. 11 and 12. Sections of six and seven somites respectively. *r*, rudimentary somite. $\times$ 157.

of cutting off somites is a rapid one, is shown in Fig. 10, in which it will be noted that the somite is completely formed before there is any indication of one succeeding it.

E. Discussion and Conclusion.

Since Miss Platt's account of the formation of the incomplete somite is not essentially different from that of mine, it follows that we differ only as regards one cleft. According to Miss Platt's view one cleft slowly forms in front of the first one, and hence one complete somite arises anterior to the first formed somite.

I have shown that this author has mistaken the most posterior of certain transitory shallow depressions in the head mesoderm for a cleft. I have also made it clear that the first few (2 or 3) clefts are not completed until six or seven pairs of somites are formed. It seems reasonable to suppose, therefore, that Miss Platt has interpreted the first cleft, during the early stages of development, as a derivative of the most posterior shallow depression. It should be added that this posterior shallow depression persists longer than the others.

Since these shallow transitory depressions are situated at regular intervals, I might suggest that they lend themselves to another interpretation, namely, as vestigial clefts separating the cephalic mesoblastic somites. Notwithstanding the fact that Locy ('95) and his followers minimize the value of myotomes in ascertaining the metamerism of the vertebrate head and use neuromeres as the *sine qua non* for determining primitive segmentation, nevertheless the glimpses one gets of such structures as that cited above should not be overlooked. In fact, if these vestigial clefts are studied in connection with the various conditions seen in the myotomes, the above interpretation becomes evident, for in passing backwards from the anterior end of the embryo one finds that the clefts become more and more pronounced. This is evidenced by (1) the vestigial clefts, (2) the rudimentary somite, whose anterior cleft fails to separate it from the head mesoderm, (3) the slowness of the first clefts in cutting off the anterior protovertebræ, (4) and finally the sharpness and rapidity with which all succeeding protovertebræ are cut off. In other words the influence of the process which has completely obliterated or greatly modified the anterior cephalic somites, gradually becomes weaker in passing posteriorly, and finally ceases altogether.

In regard to the experimental work it seems unnecessary to add to what has already been said. It was noted that an injury made, either with a glass pin or an electric needle, just anterior to the first cleft, appeared upon further incubation, in the rudimentary somite, showing beyond a shadow of doubt that no somite, except the incomplete one, is formed anterior to the first mesodermic cleft. This brings the order of the appearance of somites of the chick into harmony with the general law for the early devel-

opment of the embryo, namely, that differentiation begins at the anterior end and progresses posteriorly.

HULL ZOÖLOGICAL LABORATORY,
UNIVERSITY OF CHICAGO,
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